Enhanced Recovery and Peak Shape of Acidic and Phosphopeptides Using Waters BioResolve™ Premier 1 mm ID Columns

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Introduction

Capillary and microflow chromatography are increasingly favored in high-throughput proteomics for their enhanced sensitivity and reduced solvent consumption. However, the recovery and peak shape of acidic peptides remains challenging due to non-specific adsorption to stainless steel column hardware. MaxPeak™ Premier Columns use High Performance Surfaces (HPS) Technology to mitigate unwanted interactions between acidic analytes and column hardware. In analytical-scale chromatography, HPS technology improves recovery of acidic peptides, including phosphopeptides, and reduces the amount of column conditioning required to achieve reproducible chromatography. Here, we demonstrate the benefits of HPS technology in capillary and microflow chromatography using Waters BioResolve Premier 1 mm and 300 µm ID Columns.

Experimental Details

Sample: MassPREP™ Enolase Digest with Phosphopeptides Mix
LC: ACQUITY™ UPLC™ M-Class System
Column Temp: 60 ºC
Sample Temp: 6 ºC
Mobile Phases: 0.1% Formic Acid 100% H_2O (A) or 100% ACN (B)
Columns: BioResolve Peptide C18 RP BEH™ Column, MaxPeak Premier Technology, 1.7 µm, 300Å, (1.0 x 50 mm, 1.0 x 100 mm, or 0.3 x 50 mm)

MS System: Xevo™ G3 QToF Mass Spectrometer with low-flow ESI probe
Ionization Mode: ESI Positive
Mass Range: 50-2000 m/z
Capillary Voltage: 2.5 kV
Source Temp: 120 ºC
Desolvation Temp: 250 ºC
Desolvation Gas Flow: 350 L/Hr
Cone Gas Flow: 50 L/Hr

Improved Recovery and Peak Shape of Acidic Analytes

The BioResolve C18 RP Column exhibits high recovery and low tailing of acidic peptides on the first injection. On an equivalent stainless-steel column, low recovery and high tailing of acidic peptides is observed on the first injection. With conditioning, recovery and peak shape on the stainless-steel column improve to levels comparable to the BioResolve Premier Column.

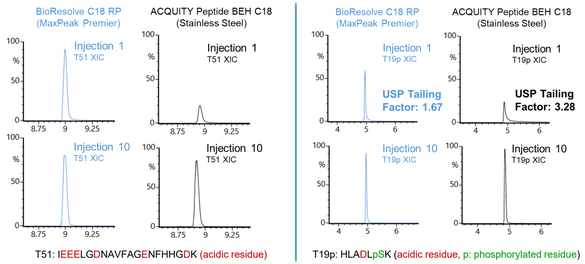

Figure 1. XICs of two acidic peptides on a Waters BioResolve C18 RP Column, MaxPeak Premier Technology (blue traces) and an ACQUITY Peptide BEH C18 Column (black traces).

Versatile Compatibility with Microflow and Analytical UHPLC Systems

Waters MaxPeak Premier 1 mm Columns can be used on both microflow and standard analytical flow UHPLC instruments. Comparable peak profiles are obtained using both the ACQUITY M-class System and ACQUITY Premier System. As expected, peak capacity for the BioResolve C18 RP 1.0 x 50 mm Column decreases on the ACQUITY Premier System due to increased impact of post-column dispersion but is comparable to Premier 2.1 x 50 mm Columns run on the same system.

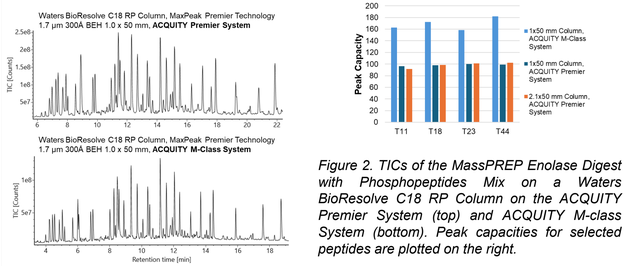

Figure 2. TICs of the MassPREP Enolase Digest with Phosphopeptides Mix on a Waters BioResolve C18 RP Column on the ACQUITY Premier System (top) and ACQUITY M-class System (bottom). Peak capacities for selected peptides are plotted on the right.

Impact of Separation Performance on MS Data Quality

Compared to three columns from alternative vendors, the BioResolve Peptide C18 RP Column delivers the cleanest mass spectra. Example spectra for the T35 peptide (WLTGPQLADLYHSLMK) are shown. On all three alternative columns, the signal for the triply charged T35 ion is over 40% lower than the BioResolve Peptide C18 RP Column. Additionally, the spectra from Columns K and H show high baseline noise across the entire mass-to-charge range analyzed. Both of these columns exhibit wide, asymmetric acidic peptide peaks, leading to co-elution of multiple species and poor signal-to-noise in the mass spectra. Less noise is exhibited in the T35 mass spectra from Column Y, but a high signal at 223.06 m/z is observed due to column bleed.

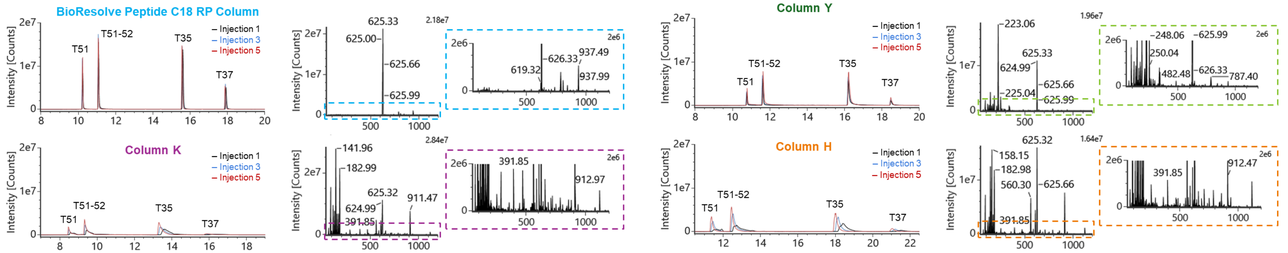

Figure 3. XICs of four acidic peptides from the MassPREP Enolase Digest with Phosphopeptides Mix on a Waters BioResolve Peptide C18 RP Column and three columns from alternative vendors. Data from injections 1, 3, and 5 are shown. The mass spectra for the T35 peptide from the first injection for each column are shown on the right.

Comparable Performance Benefits with 300 µm MaxPeak Premier Columns

Waters BioResolve Peptide 300 µm ID Columns with MaxPeak Premier Technology also deliver separation performance advantages over equivalent stainless-steel columns for capillary and low-microflow LC-MS proteomics. These columns effectively mitigate deleterious interactions between acidic peptides and column hardware, leading to improved recovery, superior peak shape, and reduced reliance on extensive column conditioning.

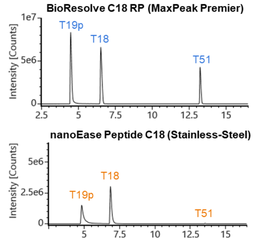

Figure 4. XICs of two acidic peptides (T19p and T51) and a non-acidic reference peptide (T18) on a Waters BioResolve Peptide C18 RP Column (top) and a nanoEase Peptide BEH C18 Column (bottom).

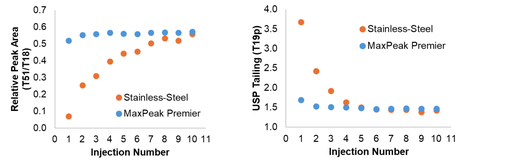

Figure 5. The relative peak area of the T51 peptide (left) and the USP tailing factor of the T19p peptide (right) as a function of injection number on a Waters BioResolve Peptide C18 RP Column and a nanoEase Peptide BEH C18 column.

Conclusions and References

The BioResolve BEH C18 RP MaxPeak Premier Technology 1 mm and 300 µm ID Columns deliver improved acidic peptide recovery and peak shape from the first injection, reducing the need for conditioning. Improved chromatographic separation leads to cleaner MS data compared to similar reversed phase columns from alternative vendors.

1. Hanna, C. M., Koza, S. M., Addepalli, B. Enhanced Recovery and Peak Shape of Acidic Peptides with BioResolve™ 1 mm ID Columns with MaxPeak™ Premier Technology. 2026, Waters App Note 720009231.
2. Hanna, C. M., Koza, S. M., Addepalli, B. Benchmarking Resolution and Recovery of BioResolve™ 1 mm ID C18 RP Columns with MaxPeak™ Premier Technology. 2026, Waters App Note 720009274.